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13. ABSTRACT ***(Maximum 200 Words)***

New contrast-specific imaging modalities such as harmonic imaging (HI) may improve the accuracy of breast ultrasound. Unfortunately, HI suffers from reduced blood-to-tissue contrast resulting from second harmonic generation and accumulation in tissue. As an alternative we propose using subharmonic imaging (SHI) by transmitting at the double the resonance frequency ($2f_o$) and receiving at the subharmonic (f_o). SHI has the potential to detect slow, small volume blood flow associated with tumor neovascularity, making early detection and identification of tumors very likely. Hence, the current project proposes to increase the ability of breast ultrasound to differentiate between benign and malignant lesions by combining injection of an ultrasound contrast agent with SHI.

To date, a dual-transducer pulse-echo system was built to perform in vitro SHI measurements and experiments were conducted using the contrast agent Optison in a perfusion phantom with realistic neovascular flow velocities (2 mm/s). Up to 12 dB of subharmonic signal components were measured. It was discovered that the Logiq 700 scanner targeted for implementation of SHI did not have the appropriate hardware configuration for running the software. A completely new Logiq 700 scanner was acquired from GE (free of charge). Three new iterations of SHI software have been developed and initial testing has commenced.

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3. TABLE OF CONTENTS

4. INTRODUCTION

The goal of any breast imaging modality is to improve the early detection of tumors and to improve the differentiation between benign and malignant lesions. While x-ray mammography is efficacious in diagnosing a high percentage of breast masses, it also produces a high rate of false positives [1]. The percentage of breast biopsies that are actually malignant vary between 10 % and 35 %. Thus, a technique that reliably differentiates between malignant and benign masses would improve the diagnosis of breast cancer and should, therefore, reduce the number of negative biopsies as well as the trauma of the patients. This proposal will attempt to establish such a technique through the novel and innovative use of subharmonic ultrasound contrast imaging.

Ultrasound imaging is currently an auxiliary modality in breast imaging. It is mainly used to differentiate between cystic and solid lesions [2]. Investigations into the possibility of breast cancer diagnosis based on Doppler ultrasound flow detection have produced mixed results, due to overlap between flow measurements in benign and malignant tumors [3-4]. One problem may be the lack of sensitivity in flow detection in small tumor vessels using ultrasound. This hypothesis is supported by reports in the pathology literature describing angiogenic vascular morphology as an independent predictor of metastatic disease [5].

Ultrasound contrast agents produce increases of 15 to 25 dB in the echo intensities of blood flow signals; especially when combined with new contrast-specific imaging modalities such as harmonic imaging [6-7]. However, harmonic imaging has been found to suffer from reduced blood-to-tissue contrast resulting from second harmonic generation and accumulation in tissue. As an alternative we propose using subharmonic imaging (SHI) by transmitting at the double the resonance frequency ($2f_o$) and receiving at the subharmonic (f_o). SHI has the potential to detect slow, small volume blood flow associated with tumor neovascularity, making early detection and identification of tumors very likely. SHI should have much better lateral resolution due to the higher transmitting frequency and should allow tumor perfusion, a measure of angiogenesis, to be estimated via time-dependent subharmonic fractional blood volume estimates. Hence, the current project proposes to increase the ability of breast ultrasound to differentiate between benign and malignant lesions by using SHI.

Quantifiable parameters of tumor angiogenesis will be estimated from the subharmonic signal intensities. A pulse-echo system will be built to perform SHI and tested in vitro as well as in vivo (in animals). The ability of SHI to depict normal vascularity as well as tumor angiogenesis will also be assessed in rabbits. Currently, the NIH and DOD have funded a study at Thomas Jefferson University into the efficacy of ultrasound contrast in the diagnosis of breast disease. We propose to expand on that project by adding SHI in the third year of this proposal. Not only is the potential of SHI in itself innovative, but because of the NIH/DOD funded study it will be possible to compare a number of new and unique approaches to breast cancer diagnosis i.e., SHI, 2D power Doppler with and without contrast as well as harmonic imaging directly to x-ray mammography. Furthermore, this project is extremely cost-effective because the existing grants coves a majority of the personnel costs as well as all major equipment purchases. The amalgamation of the NIH/DOD project with the current proposal also allows for basic research

into the correlation between SHI flow signals and pathologically detected lesion vascularity. This will enable a deeper understanding of the relationship between tumor neovascularity and ultrasound flow measurements

Consequently, this project proposes the development of a novel contrast specific imaging mode called SHI and the derivation of quantitative tumor angiogenesis estimates from SHI data. The fundamental hypothesis is that the neovasculature of malignant lesions can be visualized and quantified with SHI, thus, improving the diagnosis of breast cancer.

5. BODY

The central hypothesis of this project is that the differentiation between benign and malignant breast lesions can be improved by detection and estimation of tumor neovascularity using contrast enhanced SHI. To investigate this hypothesis SHI will be investigated in vitro and then in vivo in rabbits with VX-2 tumors. Finally, approximately 50 women with breast lesions will be recruited in year three and imaged using contrast enhanced SHI. The specific tasks of the project (as presented in the original Statement of Work) can be found in Appendix I.

First an outline of the methods applied will be given followed by a presentation of the results to date. Finally, the conclusions and future directions of the research will be discussed.

5.1 Methods

In Vitro experiments

A pulse-echo system was built to perform SHI and measure the FBV as a function of time (Fig. 1). The setup consists of a pair of confocally positioned broadband focused transducers (diameter: 2.54 cm). A pulse/function generator (8111A; Hewlett-Packard Company, Polo Alto, CA, USA) was used to generate 32 cycle busts with a PRF ranging between 20 and 100 Hz. An RF power amplifier (A150; ENI Technology Inc., Rochester, NY, USA) amplified this signal by 55 dB to generate pressure levels from 0.3 to 1.5 MPa. The transmitting transducer used was a 2.54 cm spherical focused, narrow bandwidth, 5 MHz transducer (13-0508S; Harisonic / Staveley Industries Plc, Croyden, UK). The backscattered signals were picked up using a wide band 2.54 cm spherical focused transducer with center frequency of 2.25 MHz (13-0208R; Harisonic / Staveley Industries Plc, Croyden, UK). This substantially improved the spatial resolution of the system, because scattered signals only come from the microbubbles in the small confocal region of the two transducers (1-4 mm^3 for 2 MHz transmission). The sampling frequency was 20 MHz.

The contrast agent used for this part of the study was Optison® (manufactured by Mallinckrodt Inc, St. Louis, MO, USA and co-promoted by Amersham Health, Oslo, Norway). Optison is approved for use in echocardiography by the U.S. Food and Drug administration (for improved endocardial border delineation). It consists of a suspension of perfluoropropane-filled albumin microspheres with a concentration of 6.3×10^8 bubbles/ml and the bubbles have mean diameters in the range of 3 to 5 μm. The frequency of insonation used was 4 MHz, as the resonance frequency of Optison is around 2 MHz and this is the frequency range used in the ultrasound

scanner employed for our preliminary SHI work [8-9]. This is in keeping with the concept of minimum threshold for subharmonic generation when the insonation frequency is twice the resonance frequency. The tubes used to set up the flow system were polyester shrink tubes (Advanced Polymers Inc., Salem, NH, USA) of two different diameters. The smaller tube had internal diameter of 300 μm ± 25 μm, and wall thickness of about 6 μm and the larger one had internal diameter 1 mm ± 25 μm and similar wall thickness. In this report, only results from the smaller tube will be presented, as these are the most interesting for tumor neovascularity evaluations (and results from the larger tube were presented in the previous report).

In order to simulate conditions similar to the angiogenic blood flow in tumors, we used a high efficiency, single use dialysis cartridge (F7NR; Fresenius, Bad Homburg, Germany) as a flow phantom (similar to the work by Hindle and Perkins [10]). This cartridge consists of a few thousand very thin tubes encased in a longer plastic tube. The internal diameter of each of these the thin tubes is 200 μm and they have a wall thickness of 6 μm. An acoustic window was cut into the outer tube to prevent any attenuation of the applied acoustic power.

The experimental setup for the dialysis cartridge was similar to Figure 1. The transducers were focused on the hollow tubes directly through the acoustic window of the dialysis cartridge. The cartridge and the entire setup were immersed in water. Care was taken to remove all air bubbles trapped in between the tubes; however, complete removal could not be guaranteed.

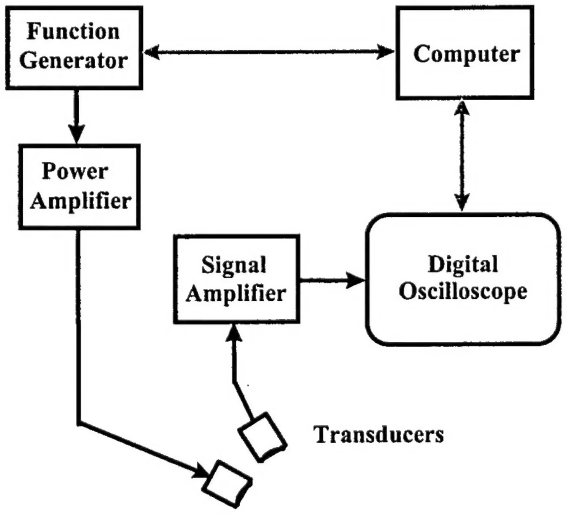

Figure 1. *The dual-transducer pulse-echo system built to perform SHI. A function generator produces a sequence of transmit pulses which are first amplified and then supplied to a single-element broadband focused transducer. Another broadband focused transducer (confocally positioned to the first transducer) will sense signals scattered from the contrast bubbles. The received signals, after being amplified, will be digitized using a digital oscilloscope. The digitized signals are further processed to obtain the subharmonic amplitude (with a bandpass filter) using LabView® (National Instruments, Austin, TX).*

A high pass filter (Krohn-Hite with a cut off at 2.3 MHz) was used at the input side, before the power amplifier to reduce any 2 MHz side bands that may be present. Its effectiveness was confirmed by the absence of any subharmonic component in the backscattered echo from water with varying acoustic pressures. The flow velocity through the hollow tubes of the dialysis cartridge was estimated to 2 mm/s. Backscattered echoes from water and contrast agent were acquired, and the subharmonic components calculated using Matlab.

In Vivo experiments

As a first step towards the animal experiments, scheduled for year 2, we installed an early version of software for SHI (as used in [9]) on the Logiq 700 scanner (GE Medical Systems, Milwaukee WI) owned by the Department. However, this software did not function correctly - in spite of having worked previously on a research Logiq 700 scanner on loan to the Department. After some investigation, it was established that the Logiq 700 scanner owned by the Department was an older model, which did not have the appropriate hardware configuration for running the SHI software. Moreover the upgrade required to run SHI on this unit was so extensive that a completely new scanner was needed.

The list price for a new Logiq 700 scanner is approximately 180,000 $, which clearly is beyond the funding available in the grant. Hence, extensive negotiations were conducted between the Department and GE Medical Systems to solve this problem. After 5 months of negotiations GE agreed to replace the existing Logiq 700 with a completely new Logiq 700 scanner free of charge to support this project. To date three new iterations of SHI software have been developed and initial testing has commenced. While it is obviously encouraging that GE has such faith in the development of SHI, the entire process became the major focus of the second half of Year 2 somewhat limiting the progress made on the project in the second year.

5.2 Results and Discussion

Figure 2 shows a comparison of the average subharmonic backscatter from the contrast agent Optison and from distilled water in a single 300 μm tube at an insonation power of 1.5 MPa. Subharmonic signals from the contrast medium could be observed over a range of frequencies around 2 MHz, with the peak signal close to 2 MHz. The amplitude of the subharmonic backscatter was about 10 dB higher than the signal from distilled water at this pressure level. Figure 3 show the subharmonic signal amplitudes (± one standard deviation) detected for insonation pressure amplitudes from 0.3 to 1.5 MPa. For comparison the average backscatter measured from distilled water flowing through the tube is also provided. Only at pressure levels above 0.9 MPa is marked subharmonic signal components measured, with the largest being approximately 10 dB above that of the water.

An important factor, which may affect the subharmonic amplitude, is the velocity of the contrast agent within the tube. The flow velocity in the 300 μm tube was about 12 cm/s. This is much higher than the expected flow velocities in the neovessels feeding tumors. The flow velocity in the dialysis cartridge was estimated at 2 mm/s, by determining the volumetric flow rate through the cartridge and dividing by the total cross-sectional area of all the thin tubes. This velocity is close to the actual flow velocity that one might expect to see in neovessels. Figure 4 shows a comparison of subharmonic backscatter amplitude from distilled water and contrast agent. An increase in the subharmonic backscatter of about 12 dB is observed when the insonation pressure

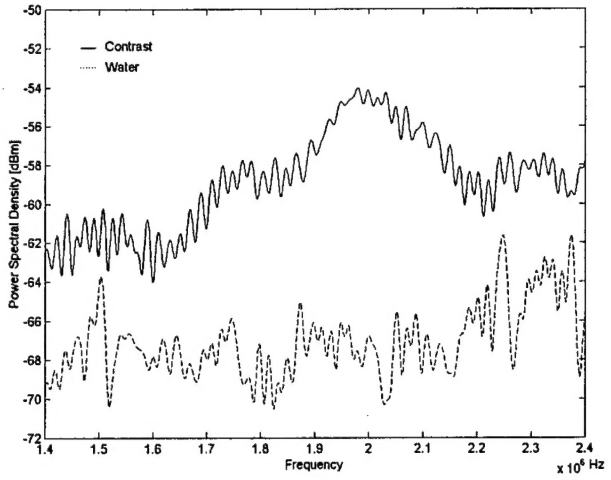

Figure 2. *Power spectral densities averaged over 16 waveforms. The waveforms correspond to backscatter from contrast agent and distilled water, flowing through a tube with internal diameter of 300 μm. The insonation power was 1.5 MPa with a PRF of 50 Hz and insonation frequency of 4 MHz.*

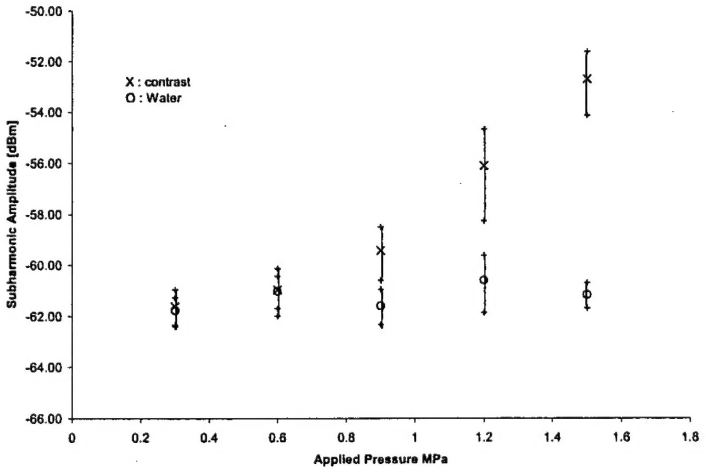

Figure 3. *The average subharmonic backscatter from the contrast agent Optison and from water as a function of the applied pressure. The tube diameter is 300 μm. The vertical bars indicate ± one standard deviation.*

was increased from 0.3 MPa to 1.5 MPa similar to the subharmonic signal components measured in the single 300 μm tube (cf., Fig. 3). This work has been submitted to the journal Ultrasonics for publication [11].

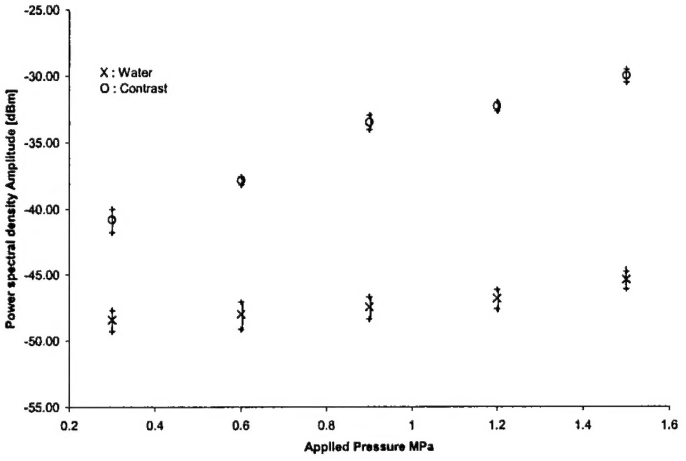

Figure 4. *The average subharmonic backscatter from Optison and from water as a function of the applied pressure in the dialysis cartridge. The internal diameter of the tubes in the cartridge is 200 μm. The vertical bars indicate ± one standard deviation.*

The current version of SHI software installed on the new Logiq 700 scanner is initially being tested on an in vitro flow phantom (ATS Laboratories, Bridgeport, CT). In Figure 5 examples of conventional ultrasound imaging (Figs. 5a and b) and SHI (Figs. 5c and d) obtained in an 8 mm vessel with a broad bandwidth curve-linear array (the 348c probe; bandwidth 2 - 4 MHz) are presented. In SHI mode pulses were transmitted at 4.4 MHz and received at 2.2 MHz. The contrast agent Optison was administrated at a dose of 1 μl/ml of water and the flow rate was kept constant at 410 ml/min. The superior suppression of tissue echoes in SHI mode is evident even before contrast administration (compare Figs 5a and c). Moreover, the enhancement produced (cf., Figs. 5b and d) appears very similar. These images represent the commencement of task 2a in the original statement of work (see the Appendix).

6. KEY RESEARCH ACCOMPLISHMENTS

- A perfusion phantom based on 200 μm tubes within a dialysis cartridge was designed.
- Velocities of 2 mm/s (i.e., capillary velocities) were realized in the perfusion phantom.
- SHI experiments were conducted with Optison in the perfusion phantom.
- Up to 12 dB of enhancement was measured for a 1.5 MPa pressure.
- A new scanner platform was acquired for SHI.
- Initial experiments for optimal SHI were performed.

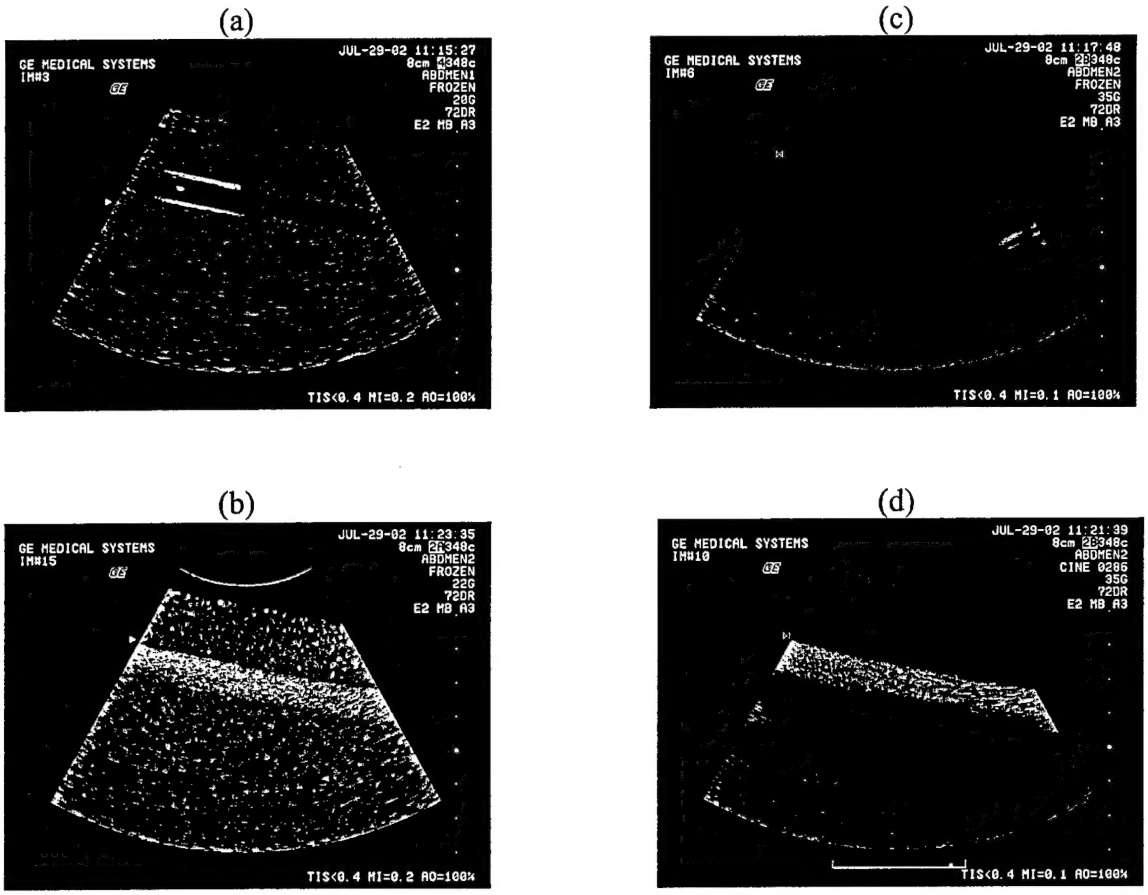

Figure 5. *in vitro conventional ultrasound imaging pre (a) and post (b) injection of Optison into a flow phantom. Also SHI acquired pre (c) and post (d) contrast. In SHI mode the transmission frequency was 4.4 MHz and the receive frequency was 2.2 MHz.*

7. REPORTABLE OUTCOMES

Govind Bhagavatheeshwaran obtained a Master of Science degree in Biomedical Engineering from Drexel University with P.M. Shankar and F. Forsberg (the PI) as his supervisors.

G Bhagavatheeshwaran, WT Shi, F Forsberg, PM Shankar. Subharmonic generation from contrast agents in simulated neovessels. Submitted to *Ultrasonics*, May, 2002.

8. CONCLUSIONS

A dual-transducer pulse-echo system was built to perform in vitro SHI measurements and experiments were conducted using the contrast agent Optison in a perfusion phantom with realistic neovascular flow velocities (around 2 mm/s). Up to 12 dB of subharmonic signal

components were measured (Fig. 4). This work has been submitted for publication in an international journal.

It was discovered that the Logiq 700 scanner targeted for implementation of SHI did not have the appropriate hardware configuration for running the software. After 5 months of negotiations GE agreed to replace the existing scanner with a completely new Logiq 700 scanner free of charge to support this project. To date three new iterations of SHI software have been developed and initial testing has commenced (Fig. 5). This development and its successful resolution became the major focus of the second half of Year 2 somewhat limiting the progress made on the project in the second year.

In summary, tasks 1a and 1b has been completed while tasks 1c and 2a are ongoing, but due to the delay in obtaining the hardware platform required the project is approximately 10 months behind schedule.

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Appendix I

The Statement of Work from the original proposal:

Objectives 1 - 2
Task 1: Software development and *in vitro* experiments (months 1 - 24)

a. Develop software for SHI and for FBV estimates to be produced from SHI data (months 1 - 24).
b. Design and implement pulse echo SHI setup (months 1 - 6).
c. Perform *in vitro* flow phantom experiments comparing SHI and FBV estimates to absolute perfusion and flow rates (months 6 - 12).

Objectives 2 - 3
Task 2: Animal experiments and data collection (months 13 - 24)

a. Perform *in vivo* experiments in 12 normal rabbits comparing FBV estimates to absolute flow rates and perfusion obtained with colored microspheres (months 13 - 20).
b. Perform *in vivo* experiments in 6 rabbits with renal VX-2 tumors implanted comparing FBV estimates to absolute tumor perfusion obtained with colored microspheres (months 20 - 24).
c. Evaluate the performance of SHI in the detection of rabbit VX-2 tumors compared to conventional ultrasound imaging, with and without contrast administration, as well as to harmonic imaging (months 13 - 24).

Objectives 4 - 5
Task 3: Human data collection and analysis (months 25 - 36)

a. Recruit 50 - 75 patients, which is about two-thirds of the anticipated number of patients being enrolled in the existing NIH/DOD supported contrast study (months 25 - 36).
b. Perform SHI contrast studies as part of the already funded NIH/DOD project. This involves an extra injection of contrast (within the permitted total dose) and will add no more than 20 minutes to the total duration of the contrast study (months 25 - 36).
c. Research coordinator to collect clinical information, pathology results, etc. (months 25 - 36).
d. Incorporate SHI findings into the existing database developed for the NIH/DOD supported study (months 25 - 36).
e. Perform ROC analysis in collaboration with the statistician (months 30 - 36).
f. Perform remaining statistical analysis in collaboration with the statistician (months 30 - 36).